Indoor Mold Exposure Associated with Neurobehavioral and Pulmonary Impairment: A Preliminary Report

KAYE H. KILBURN
University of Southern California
Keck School of Medicine
Environmental Sciences Laboratory
Alhambra, California

ABSTRACT. Recently, patients who have been exposed indoors to mixed molds, spores, and mycotoxins have reported asthma, airway irritation and bleeding, dizziness, and impaired memory and concentration, all of which suggest the presence of pulmonary and neurobehavioral problems. The author evaluated whether such patients had measurable pulmonary and neurobehavioral impairments by comparing consecutive cases in a series vs. a referent group. Sixty-five consecutive outpatients exposed to mold in their respective homes in Arizona, California, and Texas were compared with 202 community subjects who had no known mold or chemical exposures. Balance, choice reaction time, color discrimination, blink reflex, visual fields, grip, hearing, problem-solving, verbal recall, perceptual motor speed, and memory were measured. Medical histories, mood states, and symptom frequencies were recorded with checklists, and spirometry was used to measure various pulmonary volumes and flows. Neurobehavioral comparisons were made after individual measurements were adjusted for age, educational attainment, and sex. Significant differences between groups were assessed by analysis of variance; a *p* value of less than 0.05 was used for all statistical tests. The mold-exposed group exhibited decreased function for balance, reaction time, blink-reflex latency, color discrimination, visual fields, and grip, compared with referents. The exposed group's scores were reduced for the following tests: digit-symbol substitution, peg placement, trail making, verbal recall, and picture completion. Twenty-one of 26 functions tested were abnormal. Airway obstructions were found, and vital capacities were reduced. Mood state scores and symptom frequencies were elevated. The author concluded that indoor mold exposures were associated with neurobehavioral and pulmonary impairments that likely resulted from the presence of mycotoxins, such as trichothecenes.
<Key words: balance impairment, cognitive impairment, indoor air, mycotoxins, *Stachybotrys atra*, volatile organic chemicals>

THE ENERGY CRISIS OF THE 1970s, which resulted in decreased importation of Middle East crude oil to the United States and Europe, stimulated energy conservation in these 2 regions. After air leaks in buildings were plugged and air exchanges decreased, many people became sick indoors. Initial reports of Sick Building Syndrome (SBS) were subsequently doubted, and building occupants were considered hysterical[1–3] or to have suffered from "crowd disease."[3] Continuing reports of respiratory symptoms, increased sensitivity to chemicals, and memory and concentration defects spurred a search for causes.[4] Volatile organic chemicals[5] and formaldehyde[6] were considered as possible causes of SBS; however, their levels correlated poorly with symptoms.[7] Some individuals who moved out of mold-contaminated buildings soon after the onset of their symptoms improved,[8] but their symptoms recurred upon their return, whereas other individuals' symptoms persisted.

Asthma and flu-like symptoms have been associated with molds that grow indoors.[9,10] People complain of odors, memory loss, lack of concentration, sleep disturbances, and depression.[10,11] Mold growth has been associated with water condensation in walls; in air ducts; and under carpets, tiles, and flooring.[9] Water has leaked from roofs, windows, water pipes, shower stalls, bathtubs, and drains. Mold spores were observed microscopically and were grown in culture from both air and surface samples.[9,10] Patients' serum immunoglobulin (Ig)G, IgM, IgE, and IgA antibodies to molds were elevated, and their saliva contained elevated IgA antibod-

ies.[10] Antibodies to aflatoxins, trichothecenes, and satratoxins occurred frequently.[11] Case recognition relied on symptoms, on molds verified microscopically, and on the basis of what grew on culture. Patients' respiratory symptoms, complaints of defective memory and concentration, impaired balance, and depression[10,11] led to measurements of lung and central nervous system functions.

Method

Study subjects. Sixty-five consecutive adult patients who consulted the author after being exposed to molds in their respective homes in California, Arizona, and Texas—a self-selected case series—were evaluated in 2001 and 2002. The patients' homes had mold growth on walls and floors, and the molds were identified microscopically. Cultures of indoor air samples frequently grew *Stachybotrys chartarum, Aspergillus, Penicillium, Chaetomium, Alternaria, Fusarium,* and *Rhizopus* at levels that exceeded concentrations found outdoors. Other fungal genera included *Actinomycetes* and *Cladosporium,* as well as the bacteria *Corynebacterium, Shigella, Agrobacterium,* and *Pseudomonas.* Each patient's serum was analyzed for antibodies to 14 categories of mold and to aflatoxin, trichothecenes, and satratoxin. Indoor air mycotoxins were not measured because methods for such measurements do not currently exist.

The 65 adult patients were compared by analysis of variance (ANOVA) with a referent group of 202 unexposed community-dwelling individuals who lived in Wickenburg, Arizona, and who were recruited and tested in 1993 and retested in 1996.[12] From this group and from comparable groups, equations for the calculation of predicted values were developed for each test.[12] The observed test values in unexposed and mold-exposed individuals were divided by the predicted values (i.e., [observed/predicted] × 100) that adjusted for sex, height, weight, and education (i.e., highest grade attained or level of higher education completed).

Reference subjects were selected at random from voter registration rolls, and individuals with occupational exposure to chemicals, and those who had medical or neurological diseases, were excluded via interview. Reference subjects comprised the largest of 3 groups, the measurements for which produced prediction equations.[12] Subjects were reimbursed for their time spent participating in this study, and all individuals provided informed consent after the procedures of the study were explained fully. The study protocol was approved by the Human Studies Research Committee of the University of Southern California, Keck School of Medicine.

Questionnaires. The subjects' questionnaires were checked by computer-guided card readings, and omissions were rectified by the subjects. The frequencies of occurrence of 35 common health complaints[13,14] were rated on a scale from *never* (1) to *daily* (11). Subjects answered the American Rheumatism Association's lupus erythematosus questions[15] and a standard respiratory questionnaire,[16] and gave histories of occupational and other exposures to chemicals, pesticides and herbicides, tobacco, alcohol, and drugs (prescription and illicit). Histories included recorded incidents of unconsciousness; anesthesia; head trauma; and medical, neurological, or psychiatric illnesses.[14,17] The questionnaires and neurophysiological and neuropsychological test batteries evolved through studies of histology technicians and firefighters, and through examinations of the effects of formaldehyde,[14,17,18] thermolysis products of polychlorinated biphenyls (PCBs),[19] and exposure to toluene-rich chemical waste.[20] These instruments also took into consideration several groups of unexposed reference subjects.[12,20]

Neurophysiological tests

Simple reaction time and visual 2-choice reaction time. Simple reaction time (SRT) and visual 2-choice reaction time (CRT) were the times from appearance to cancellation—by tapping a touch pad A or S—of a 10-cm block A for SRT and 10-cm blocks A and S for CRT, both of which were measured with a computerized instrument (Neurotest, Inc. [Pasadena, California]).[21] The lowest median score of the final 7 attempts in each of 2 trials of 20 attempts was accepted for SRT and for CRT.

Body balance. Body balance was measured while the subject was standing erect, with his or her feet aligned closely, side-by-side. The position of the head was tracked by 2 microphones from a sound-generating stylus on a headband (Neurotest, Inc. [Pasadena, California]). Results were processed by software (Graf/Bar Mark II [Scientific Accessories Corp. {Shelton, Connecticut}]) and expressed as the mean speed of sway in cm/sec.[22] The minimal sway speed was recorded for 3 consecutive 20-sec trials with eyes open, and 3 with eyes closed, in alternating sequence.

Blink reflex latency R-1. Blink reflex was measured electromyographically with surface electrodes placed on the lateral orbicularis oculi muscles bilaterally,[23,24] after the right and left supraorbital notches were tapped with a light hammer, which also triggered a recording computer (Neurotest, Inc. [Pasadena, California]). Ten R-1's were averaged for each side, and failures were recorded.[24]

Color confusion index. The color confusion index was measured with the desaturated Lanthony 15-hue test under conditions of constant illumination,[25] and the index was scored by Bowman's method.[26]

Hearing. Hearing was measured with standard programmed audiometers (Model ML-AM [Microaudio-

metrics {South Daytona, Florida}]) at stepped frequencies of 500–8,000 Hz. Results were expressed as the sum of hearing loss at each frequency for each ear.

Visual fields. For the threshold testing of visual fields, an automated, computerized perimeter recorder (Med Lab Technologies [North Wales, Pennsylvania]) that mapped the central 30° of the right and left eyes individually, was used. The performance of each eye was determined as the sum (in decibels) of the threshold of 80 points within the central 30°.

Neuropsychological testing. Immediate memory or recall was measured by memory or recall associated with information contained in 2 stories gleaned from Wechsler's Memory Scale–Revised (WMS-R).[27] Culture Fair (Scale 2 form A) tested for nonverbal, nonarithmetic intelligence, on the basis of the selection of 4 sets of designs[28,29]: logical series, difference, completion, and pattern definition and transfer. Culture Fair resembles Raven's progressive matrices.[30] The 46-word multiple-choice vocabulary test used originated from the multidimensional aptitude battery.[31] Digit symbol substitution from the Wechsler Adult Intelligence Scale–Revised (WAIS-R)[32] tested attention and integrative capacity. Information, picture completion, and similarities—all of which were also gleaned from the WAIS-R—tested for long-term (i.e., embedded or hold) memory. The time required to place 25 pegs in a grooved pegboard (from the Lafayette battery) to make trails A and B assessed dexterity, coordination, and decision-making; fingertip number writing measured peripheral perception and discrimination (from the Halstead-Reitan battery[33,34]). Subjects self-appraised their emotional status during the preceding week by using the Profile of Mood States (POMS)[35]—which comprises 65 words that describe tension, anxiety, depression, anger, vigor, fatigue, confusion—and the Limbic Check List.[36] Recall of the Rey 15 figures assessed malingering.[37]

Neurological examinations. The author conducted neurological examinations to assess cranial nerve function, muscle movement and strength, and cerebellar signs (e.g., past pointing, tremors, alternating movements, stance, and gait).

Respiratory flows and vital capacities. Respiratory flow and vital capacity were measured during a full inspiration while the subject, with a nose clip attached to his or her nose, stood upright and exhaled into a volume-displacement spirometer (Model 822 [Sensor Medics {Anaheim, California}]). The procedure was repeated until readings from 2 forced expirations comported within 5%.[38] Records were traced with a digitizer; measured with software (Graf/Bar Mark II [Scientific Accessories Corp. {Shelton, Connecticut}]), and compared with predicted values adjusted for height, age, sex, and years of cigarette smoking.[39]

Alcohol and carbon monoxide measurements. Fuel-cell analyzers (Alcohol Intoximeter [St. Louis, Missouri] and a Micro Smokerlyzer [Bedfont Scientific Ltd {Rochester, U.K.}] were used to measure alcohol and carbon monoxide, respectively, in air expired following a 20-sec breath hold.[20]

Statistical analyses. Scores and computed data were entered into an IBM-compatible microcomputer of our design. Descriptive and analytical computations—adjusted for differences in age, education, sex, height, and weight—were completed with stepwise linear-regression modeling in order to develop prediction equations for each test. Computations were completed with Stata Statistical Software, version 8 (Stata Corporation [College Station, Texas]). Prediction equations were developed from measurements of 293 subjects. Measurements had symmetrical distributions.[12] The observed measurements and scores for each patient were compared with his or her individual predicted values and expressed as percent predicted. The exposed group's percent predicted values were then compared with those of the control group, using ANOVA. Factors such as family income, hours of general anesthesia undergone, POMS score, and depression score were excluded because their coefficients were insignificant. Statistical significance was defined as $p < 0.05$. Abnormalities for the 65 patients and 202 referent subjects were counted (Table 1), after bilateral tests (e.g., hearing) were assigned a value of 0.5 per side—except for visual field performance which was assigned a value of 1 per side, and balance with eyes open and eyes closed which were scored at 2 each.

Results

All 65 patients (i.e., approximately 40 families) had observed mold growing on the walls in their kitchens, bathrooms and/or service areas, and sometimes on the bedroom and closet walls. Musty odors were traced to electrical plates and air-conditioning ducts that provided access to inner walls. Invariably, several mold genera grew in cultures of indoor and outdoor air samples obtained simultaneously; occasionally, endotoxin-producing bacteria were present. Variability of sampling and characterization methods made quantitative comparisons impractical; however, the data were correlated with the complaints, impairment profiles, and serum antibodies.

The mold-exposed group was not statistically significantly older than the unexposed group, but they had greater educational attainment. The exposed group's mean SRTs and CRTs were prolonged significantly, as evidenced by the percent predicted, compared with unexposed subjects (Table 1). With respect to balance, measured sway speeds were faster and significantly abnormal—especially with eyes closed—in the mold-exposed group. Blink reflex latency R-1 was delayed significantly. Color discrimination errors were increased.

Table 1.—Characteristics and Test Results for Mold-Exposed Subjects Compared with Referent (Unexposed) Subjects, as Percentage of Predicted

Subject characteristic/test	Exposed (n = 65) $\bar{x}$	Exposed (n = 65) SD	Unexposed (n = 202) $\bar{x}$	Unexposed (n = 202) SD	p*
Subject characteristic					
Age (yr)	47.3	13.7	46.6	20.6	0.624
Education level (yr)	14.6	2.5	12.9	2.3	0.0001
Neurophysiological test					
Reaction time (ms)					
Simple	103.8	6.6	99.9	3.7	0.0001
Choice	102.4	4.4	100.0	2.5	0.0001
Balance sway speed (cm/sec)					
Eyes open	137.0	44.6	100.2	20.0	0.0001
Eyes closed	167.4	95.3	103.1	26.7	0.0001
Blink reflex latency R-1 (ms)					
Right	114.9	13.2	99.4	14.6	0.0001
Left	115.4	12.7	96.4	13.2	0.0001
Hearing loss					
Right	105.7	38.3	101.5	24.6	0.436
Left	105.3	35.4	98.3	23.2	0.213
Color discrimination errors					
Right	72.0	42.6	102.6	51.1	0.0001
Left	74.0	49.0	102.6	51.1	0.0001
Visual field performance					
Right	87.4	21.7	100.0	22.8	0.006
Left	89.6	19.1	101.1	21.7	0.008
Grip strength					
Right	90.1	18.8	99.3	17.5	0.0001
Left	86.8	22.1	99.1	17.5	0.0001
Neuropsychological test					
Culture Fair	95.8	21.3	101.2	20.0	0.063
Digit symbol	93.5	10.4	101.5	9.2	0.0001
Vocabulary	88.6	28.6	99.2	20.8	0.015
Verbal rtecall					
Immediate	79.5	24.6	99.8	31.1	0.0001
Delayed	71.7	38.0	99.9	41.3	0.0001
Pegboard	92.2	15.7	101.8	25.7	0.001
Trails A	103.7	9.2	100.3	8.3	0.006
Trails B	102.9	9.1	100.4	7.5	0.026
Finger writing errors					
Right	99.4	7.3	100.0	7.5	0.647
Left	101.0	9.7	100.0	7.8	0.513
Information	82.6	36.1	101.5	39.4	0.0007
Picture completion	75.2	29.6	99.3	32.2	0.0001
Similarities	93.4	29.3	98.1	41.2	0.396
Total abnormalities	9.9	5.0	2.3	2.4	0.0001

Notes: $\bar{x}$ = mean, and SD = standard deviation.
*p values by analysis of variance.

Errors were modeled as the reciprocal of quadratic power, therefore abnormal observed values decreased compared with predicted scores. Visual field performance was reduced significantly in both eyes in the exposed group. Grip strength was also significantly lower in the exposed group. Hearing losses were not significantly different between the 2 groups.

The results of psychological tests revealed significantly lower scores in the mold-exposed group for digit symbol substitution and vocabulary; however, Culture Fair (i.e., problem-solving) scores were not significantly different between the 2 groups. Immediate verbal recall (of stories) was significantly lower in the exposed group, and decreased further following a 30-min delay.

Times for peg placement trail-making A and B were all significantly prolonged in the exposed group, thus reflecting perceptual motor impairment. Fingertip number writing perception was not impaired in either group.

Information and picture completion, but not similarities, were significantly different in the exposed group, despite the fact that these tests are usually unaffected by chemical exposures.[40]

In the mold-exposed group, 12 of 14 physiological functions, and 9 of 13 psychological tests, were affect-

ed adversely by molds and mycotoxins, for a total of 21 of 27 neurobehavioral tests. Mold-exposed subjects averaged 9.9 abnormal tests, compared with 2.3 among the referents ($p < 0.0001$ [Table 1]).

The mood states (i.e., POMS) scores were 3 times higher in the exposed group than in the unexposed group—a significant difference. Vigor was reduced, and tension, depression, anger, fatigue, and confusion were higher in the exposed group (Table 2). The POMS score equals the sum of the 5 adverse moods, minus vigor. Mean frequencies of 35 symptoms averaged 5.0 ± 1.9 (mean ± standard deviation) in the exposed group, compared with the controls (2.6 ± 1.1), which was a significant difference ($p < 0.0001$).

Vital capacity was reduced significantly in the mold-exposed group, as was forced expiratory volume in 1 sec ($FEV_{1.0}$)—results evidencing airway obstruction (Table 3). The decrease in vital capacity reduced the slowly emptying lung space; therefore, mid-flow forced expiratory flow (i.e., FEF_{25-75}), terminal flow (FEF_{75-85}), and the $FEV_{1.0}$-to-forced vital capacity (FVC) ratio were all higher in the exposed than the unexposed group.

Fifty-two percent of mold-exposed patients had current symptoms of peripheral neuropathy, and 62% of these patients had a history of peripheral neuropathy. This percentage compared with 14% of unexposed subjects.

Sixty-eight percent of patients had developed excessive sensitivity to chemicals (i.e., chemical triggers) following mold exposure, and 57% reacted to more than 2 chemicals. However, regression analysis for the influence of numbers of chemical triggers, age, POMS score, and other factors on total abnormalities showed that only POMS had a significant correlation coefficient ($r = .031698$, $p < 0.03$), but with little effect ($r^2 = 5.7\%$). Regression analysis for the influence of peripheral neuropathy score, age, and other factors on total abnormalities showed a correlation coefficient for peripheral neuropathy score of 0.2709596 ($p < 0.029$; $r^2 = 6.2\%$)—again, with little effect.

Table 2.—Profile of Mood States (POMS) Test Results for Mold-Exposed Subjects Compared with Unexposed Subjects

	Exposed ($n = 65$)		Unexposed ($n = 202$)		
POMS symptom	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*p*
Score*	64.0	46.1	21.0	31.6	0.0001
Tension	17.0	8.8	9.1	5.8	0.0001
Depression	15.7	13.2	8.1	9.3	0.0001
Anger	14.0	11.1	8.3	7.9	0.0003
Fatigue	16.2	8.2	7.6	6.1	0.0001
Vigor	11.1	7.1	18.3	6.3	0.0001
Confusion	13.6	7.0	6.1	4.5	0.0001
Symptom frequency	5.0	1.9	2.6	1.1	0.0001

Notes: $\bar{x}$ = mean, and *SD* = standard deviation.
*The POMS score is the sum of tension, depression, anger, fatigue, and confusion, minus vigor.

Table 3.—Pulmonary Function Test Results for Mold-Exposed Subjects Compared with Unexposed Subjects, as Percentage of Predicted

	Exposed ($n = 65$)		Unexposed ($n = 202$)		
Pulmonary function test	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*p**
FVC	91.9	11.1	101.6	15.1	0.0001
$FEV_{1.0}$	88.4	12.7	93.6	15.8	0.016
FEF_{25-75}	93.6	28.9	88.1	35.0	0.256
FEF_{75-85}	91.6	51.1	78.1	52.7	0.070
$FEV_{1.0}$/FVC	77.2	7.1	72.9	9.5	0.0007

Notes: $\bar{x}$ = mean, *SD* = standard deviation, FVC = forced vital capacity, $FEV_{1.0}$ = forced expiratory volume in 1 sec, FEF_{25-75} = mid pulmonary forced expiratory flow, and FEF_{75-85} = terminal pulmonary flow.
**p* values by analysis of variance.

The neurological abnormalities seen most frequently in mold-exposed persons were past-pointing in 48 subjects, decreased or absent vibration sensation in the feet in 35, ataxia in 32, slow alternating movements in 21, dysmetria in 16, and tremors in 12. No one had clonus or cogwheel rigidity. One third of the exposed patients had more than 4 abnormal cerebellar signs and abnormally fast sway speed with eyes closed.

Satratoxin antibodies were increased in exposed subjects by 178%, but antibodies to aflatoxin and trichothecenes were decreased by 55% and 22%, respectively. Serum antibody titers to mold showed that IgM increased by 114%, IgE by 47%, and IgG by 42% among the exposed subjects (Table 4). Serum antibody titers to molds or mycotoxins were not predictive of neurobehavioral abnormality, as evidenced by the results of regression analyses. Pulmonary impairment, as shown by the results of spirometric tests, was not correlated with mold concentrations in air, or with antibody titers.

Impairment in 6 patients exposed to additional contaminants of sewer gas, construction materials (including organic solvents), or pesticides averaged below the group mean. Excluding these individuals from analysis did not affect the interpretation; therefore, they were retained in the analysis. No patient had a neurological or psychiatric disease. Severe depression subsequent to mold exposure was diagnosed and treated in 4 patients.

Eight mold-exposed patients were tested 11–20 mo following their initial evaluations, after they had left the homes in which exposure occurred. Repeat testing showed that no individual had experienced improved functions; in fact, 7 of the 8 patients (88%) had worsened and 1 individual's symptoms were unchanged. These observations, which allow for comparison of functions across time and enable each individual to function as his or her own control, confirm comparisons of groups after the application of prediction equations. Absent additional mold exposure, functions in 88% (n = 7) of these patients had deteriorated during the course of a year.

Table 4.—Differences in Prevalence of Abnormal Titers (in %) of Antibodies to 14 Molds and 3 Mycotoxins in Mold-Exposed Patients with Symptoms and Impairments, Compared with Community (Unexposed) Subjects

Antibodies measured	Exposed (n = 54)	Unexposed (n = 48)	Difference between groups
Mold*			
IgG	74.0	52.0	> 42
IgM	94.0	44.0	> 114
IgA	33.0	79.0	< 42
IgE	117.0	79.0	> 47
Mycotoxin			
Aflatoxin	9.2	16.7	< 55
Satratoxin	40.7	22.9	> 178
Trichothecene	13.0	16.7	< 22

Note: Ig = immunoglobulin.
*The following mold antibodies were measured: *Alternaria tenus, Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor, Chaetomium globosum, Cladosporium herbarum, Epicoccum nigrum, Geotrichium candidum, Penicillium notatum, Phoma herbarum, Pullullaria pullulans, Rhizopus nigricans, Rhodotorula glutinis, Stachybotrys chartarum.*

Discussion

Many neurobehavioral measurements in 65 subjects exposed to molds and mycotoxins at home were abnormal, compared with their individual predicted values. Mold exposure was associated with impaired balance, color discrimination, visual field performance, and grip strength, as well as slowed reaction times—but *not* with decreased hearing. Verbal recall was decreased, perceptual motor functions were impaired for peg placement and trail making, and problem solving was reduced in digit symbol substitution. Vocabulary and long-term memory for information and picture completion were impaired. Excessive cerebellar abnormalities—evidenced by the results of neurological examinations—correlated with measured balance as excessive sway speed.

These mold-exposed patients complained of persistent flu-like illnesses, onset of asthma (not verified by exam), beginning of severe fatigue, impaired memory and concentration, frequent dizziness, and unsteady balance. These symptoms were accompanied by the patients noticing musty odors and black mold growth on baseboards and lower walls (particularly in the bathrooms, adjoining bedrooms, and closets) and on outer walls beneath window sashes and where walls joined ceilings. Concurrent with symptoms, water leaks were usually found from roofs, windows, outer walls, or within the walls. Microscopic examination of touch preparations showed the spores and hyphae of many different molds. Patients' serum and saliva antibodies for many molds exceeded laboratory reference values, but only satratoxin antibodies were elevated compared with an unexposed group.

Clinical judgment attributed these impairments to mold exposure, after other possibilities had been considered, inasmuch as these patients were without preexisting medical or neurological diseases or traumatic brain damage. In addition, mold-exposed patients had no previous chemical exposures and they had rarely used pesticides.

Pulmonary function abnormalities indicated severe airways obstruction that reduced vital capacity and $FEV_{1.0}$, and their reduced ratio was characteristic of bronchiolitis.[41,42] There is no accepted method for the measurement of mycotoxins in air or for the quantification of molds in air (except by spore count or culture),

and no serum assay or biomarker is available; therefore, we lack the doses for a dose-response curve of adverse effects on the brain.

Mycotoxins are recognized by bioassays for toxic activity on cells or enzymes, and trichothecene activity can be estimated in air or particles.[43–46] Dust samples from a Montreal, Canada, office building were assayed by thin-layer chromatography, using 4-(*p*-nitrobenzyl) pyridine to identify the 12,13-epoxy group of trichothecenes.[44] Methanol extractions of water-damaged building materials and gypsum board, assayed in feline skin and lung cells, were 200 times as toxic as board extract that was not water damaged.[44] Spores and fragments of 3 molds (i.e., *Aspergillus versicolor, Penicillium melinii,* and *Cladosporium cladosporioides*) share antigens and immunological reactivity.[45] A trichothecene assay (i.e., an inhibition of protein synthesis by using firefly luciferase translation in rabbit reticulocytes) correlated with fungal counts in indoor air.[47]

Trichothecenes identified by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) cleavage, and shown chemically by high-performance liquid chromatography with diode array ultraviolet detection and gas chromatography-mass spectrometry, were cytotoxic for swine kidney cell cultures.[46] A chymotrypsin-like serin proteinase from *Stachybotrys chartarum* was isolated and purified from an infant with pulmonary hemosiderosis. It cleaved lung proteinase inhibitors, bioactive peptides, and collagen,[48] all of which suggested that it could destroy lung tissue.

The consecutive mold-exposed patients in the present study were referred by physicians or attorneys, or were self-referred. A representative study of a random sample of individuals would necessitate motivating individuals who had no complaints to submit to testing. Such a study might be feasible when mold problems are better understood and a biological marker is available.

Patients' exposures to toxins at home are a difficult epidemiological problem. They are not a cohort with shared space and exposure, as is the case in a workplace. Age, wellness, and hours per day of exposure vary more for people at home than for individuals at work. Self-selection for testing (bias) may ultimately result in an increase in symptoms, but it does not affect neurobehavioral measurements.[49,50] Measurements of effects are independent, even if symptoms are exaggerated. Clinical judgment has favored molds as causes, although homes—like schools and offices[8,9,42]—often contain other toxic chemicals.[6,7]

Impairment from mold exposure resembles that associated with formaldehyde exposure and the Indoor Air Syndrome—except for the lengthened blink reflex latency[6] that is typically associated with exposure to chlorinated solvents and arsenic,[49–51] but not with other chemicals.[52–54]

Antibody titers to trichothecenes, satratoxin, and aflatoxin did not predict impairment of lung or brain. An important relationship to consider in future studies is that which may exist between total and specific neurobehavioral abnormalities, and trichothecene concentrations—especially T-2 and satratoxin—in indoor air[47,55] and in patients' serum and fat.

Observations of cognitive and memory impairment in 20 previous patients exposed to *Stachybotrys atra,*[56] as well as abnormalities of balance, reaction time, recall, memory, and trail making in our preliminary group[11] are now confirmed.

Inhaled mycotoxins, liberated from indoor mold growth, caused brain impairment and neurological symptoms. Trichothecenes are epoxides that covalently adduct deoxyribonucleic acid, ribonucleic acid, protein, and microtubules of nerve axons,[56,57] providing mechanisms for lung, brain, and immune system toxicity.

Most therapeutic antibiotics, including penicillin and aminoglycosides, are products of molds[58] Other drugs produced by molds include ergot alkaloids, coumadins, and adriamycin C—used to treat cancer.[59] Asthma, cancer, wasting, hemorrhage, inanition, and death have been linked to mold exposure since ancient times.[10,59] But why have people developed sickness from molds in their homes during the most-recent decade?

Consider that after World War II construction of the interior walls of homes shifted from wood or metal lath, plaster, and lime coat to plasterboard (gypsum board)—a change that reduced cost. The inner paper (cellulose) layer of plasterboard encourages mold growth[60] when inner walls become damp from inadequate venting of moisture or from leaks from walls, roofs, or plumbing.[61] Variations in moisture levels stress molds to produce spores and toxins, such as trichothecenes and satratoxin,[62,63] that escape into living and working spaces.[43] Prior to the use of plasterboard, alkaline plaster or lime coating on walls discouraged fungal growth.

There are no antidotes for mold toxins.[59,62] They are likely to permanently impair brain function. Only the avoidance of exposure stops added damage; however, impairment may progress *absent additional exposure*—as was the case in 7 of 8 patients we tested twice—suggesting that *absorbed dose continues to damage neurons.*

Prevention

Limiting the damaging health effects of mycotoxin exposure requires primary prevention. An initial step is the development of wallboard that does not facilitate the growth of *Stachybotrys* and other toxic molds. Perhaps boric acid or sodium borate can be added to the paper layer or slurry of gypsum during the making of wallboard. Other agents that provide an acidic milieu when applied to surfaces may prevent mold growth; methoxymethane (dimethylether) removes aflatoxins from peanuts.[64] It is important, however, that any agent

developed for the lessening of mold exposure not be toxic itself. Recognition by the medical profession and by the public that mold causes neurological and/or respiratory damage is important because these illnesses are often progressive and irreversible. The social, personal, and economic consequences are immense. Prevention by avoidance of exposure is imperative.

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Requests for reprints should be sent to Dr. Kaye H. Kilburn, University of Southern California, Keck School of Medicine, Environmental Sciences Laboratory, Bldg. A7 #7401, 1000 S. Fremont Ave, Unit 2, Alhambra, CA 91803.

E-mail: Kilburn@usc.edu

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